

WHAT IS CLAIMED IS:

5           1. A peptide composition immunoreactive with  
antibodies to a native protein wherein the peptide  
comprises an amino acid sequence of six to 50 amino  
acids, and the sequence comprises two Cys residues which  
are separated from each other by at least about two but  
fewer than twenty non-Cys amino acid residues and wherein  
10          thiol groups of the Cys residues are reversibly protected  
by chemically reversible means.

          2. The peptide of claim 1, wherein the Cys  
residues are protected from oxidation by ethylcarbamoyl,  
15          acetamidomethyl, 3-nitro-2-pyridinesulfinyl or diphenyl-  
4-pyridylmethyl.

          3. The peptide of claim 2, wherein the Cys  
residues are protected from oxidation by ethylcarbamoyl.  
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          4. The peptide of claim 1, further comprising  
a Cys residue at the N-terminus which is not protected  
from oxidation.

25          5. The peptide of claim 4, wherein the N-  
terminal amino acids comprise Cys-Gly-Gly.

          6. The peptide of claim 4, wherein the C-  
terminus amino acid is amidated.  
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          7. The peptide of claim 1, wherein the Cys  
residues are separated from each other by about four to  
six non-Cys residues.

35          8. The peptide of claim 1 which is  
immunoreactive with antibodies to a retroviral  
transmembrane protein.

9. The peptide of claim 8, wherein the retroviral protein is HIV-1 gp41 and the peptide comprises at least seven contiguous amino acids within the following sequence:

Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-  
Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-  
Lys-Leu-Ile-Cys.

10. The peptide of claim 9, wherein the N-terminus comprises amino acids added to enhance immunospecific reactivity, wherein at least one of said additional amino acids is a Cys residue not protected from oxidation.

11. The peptide of claim 10, wherein the Cys not protected from oxidation is the N-terminal residue.

12. The peptide of claim 11, wherein the N-terminus sequence is Cys-Gly-Gly.

13. The peptide of claim 11, wherein the C-terminus amino acid is amidated.

14. The peptide of claim 8, wherein the retroviral protein is HIV-2 gp36 and the peptide comprises at least seven contiguous amino acids within the following sequence:

Arg-Val-Thr-Ala-Ile-Glu-Lys-Tyr-Leu-Gln-Asp-  
Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys-Ala-Phe-  
Arg-Gln-Val-Cys.

15. The peptide of claim 14, wherein the N-terminus comprises amino acids added to enhance immunospecific reactivity, wherein at least one of said

additional amino acids is a Cys residue not protected from oxidation.

16. The peptide of claim 15, wherein the Cys not protected from oxidation is the N-terminal residue.

17. The peptide of claim 16, wherein the N-terminus sequence is Cys-Gly-Gly.

18. The peptide of claim 16, wherein the C-terminus amino acid is amidated.

19. The peptide of claim 8, wherein the retroviral transmembrane protein is HTLV-I gp21 and the peptide comprises at least about seven contiguous amino acids within the following sequence:

Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Trp-  
Glu-Gln-Gly-Gly-Leu-Cys-Lys-Ala-Leu-Gln-Glu-  
Gln-Cys.

20. The peptide of claim 19, wherein the N-terminus comprises amino acids added to enhance immunospecific reactivity, wherein at least one of said additional amino acids is a Cys residue not protected from oxidation.

21. The peptide of claim 20, wherein the Cys not protected from oxidation is the N-terminal residue.

22. The peptide of claim 21, wherein the N-terminus sequence is Cys-Gly-Gly.

23. The peptide of claim 21, wherein the C-terminus amino acid is amidated.

24. The peptide of claim 8, wherein the retroviral protein is HTLV-II gp21 and the peptide comprises at least seven contiguous amino acids within the following sequence:

Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Trp-  
Glu-Gln-Gly-Gly-Leu-Cys-Lys-Ala-Ile-Gln-Glu-  
Gln-Cys.

25. The peptide of claim 24, wherein the N-terminus comprises amino acids added to enhance immunospecific reactivity, wherein at least one of said additional amino acids is a Cys residue not protected from oxidation.

26 The peptide of claim 25, wherein the Cys not protected from oxidation is the N-terminal residue.

27. The peptide of claim 26, wherein the N-terminus sequence is Cys-Gly-Gly.

28. The peptide of claim 26, wherein the C-terminus amino acid is amidated.

29. A method for preparing a peptide coated solid phase for immunological detection and/or quantitation of antibody to a protein, comprising:

(a) synthesizing a peptide which comprises an amino acid sequence of six to 50 amino acids and having two Cys residues which are separated from each other by at least about two but fewer than twenty non-Cys amino acid residues;

(b) protecting thiol groups of the cysteine encoded within the peptide sequence by chemically reversible means to form a protected peptide composition;

(c) immobilizing the protected peptide composition on a solid phase;

(d) removing the chemically reversible protection means from the immobilized peptide composition;

(e) incubating the immobilized peptide composition under conditions conducive to the formation of disulfide bonds.

30. The method of claim 29, wherein the cysteine thiol groups are protected prior to synthesis of the peptide sequence.

31. The method of Claim 29, wherein the chemically reversible protection means is ethylcarbamoyl, acetamidomethyl, 3-nitro-2-pyridinesulfinyl or diphenyl-4-pyridylmethyl.

32. The method of Claim 31, wherein the chemically reversible protection means is ethylcarbamoyl.

33. The method of Claim 29, wherein the peptide is immobilized by adsorption.

34. The method of Claim 29, wherein the peptide is immobilized to the solid phase by covalent attachment.

35. The method of Claim 29, wherein the solid phase is latex, silica, cellulose, fluorocarbon polymers, nylon, polyacrylamide or polystyrene.

36. The method of Claim 29, wherein the solid phase is a latex of silica, cellulose, polyacrylamide or polystyrene.

37. The method of Claim 35, wherein the solid phase is latex of polystyrene.

38. The method of claim 29, wherein the peptide sequence is:

Y-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-  
Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys\*-Ser-Gly-  
Lys-Leu-Ile-Cys\*-X,

Y-Arg-Val-Thr-Ala-Ile-Glu-Lys-Tyr-Leu-Gln-Asp-  
Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys\*-Ala-Phe-  
Arg-Gln-Val-Cys\*-X,

Y-Gln-Asp-Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys\*-  
Ala-Phe-Arg-Gln-Val-Cys\*-X,

Y-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Trp-  
Glu-Gln-Gly-Gly-Leu-Cys\*-Lys-Ala-Leu-Gln-Glu-  
Gln-Cys\*-X, or

Y-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Trp-  
Glu-Gln-Gly-Gly-Leu-Cys\*-Lys-Ala-Leu-Gln-Glu-  
Gln-Cys\*-X,

wherein X is OH or NH<sub>2</sub>, Y comprises amino acids added to  
enhance the reactivity and Cys\* is a cysteine residue  
comprising a thiol group protected by chemically  
reversible means.

39. A method for determining the presence of  
antibodies to HIV viruses or antigen of HIV viruses in a  
body fluid, comprising:

(a) contacting under conditions which permit  
immobilization, a solid phase and a composition  
containing at least one peptide comprising the amino acid  
sequence:

Y-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-  
Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys\*-Ser-Gly-  
Lys-Leu-Ile-Cys\*-X,

5 Y-Arg-Val-Thr-Ala-Ile-Glu-Lys-Tyr-Leu-Gln-Asp-  
Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys\*-Ala-Phe-  
Arg-Gln-Val-Cys\*-X,

10 Y-Gln-Asp-Gln-Ala-Arg-Leu-Asn-Ser-Trp-Gly-Cys\*-  
Ala-Phe-Arg-Gln-Val-Cys\*-X,

Y-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Trp-  
Glu-Gln-Gly-Gly-Leu-Cys\*-Lys-Ala-Leu-Gln-Glu-  
Gln-Cys\*-X, or

15 Y-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Trp-  
Glu-Gln-Gly-Gly-Leu-Cys\*-Lys-Ala-Leu-Gln-Glu-  
Gln-Cys\*-X,

20 wherein X is OH or NH<sub>2</sub>, Y comprises amino acids added to  
enhance the reactivity of the peptides and Cys\* is a  
cysteine residue comprising a thiol group protected by  
chemically reversible means;

(b) removing the chemically reversible  
25 protective means from the cysteine thiol groups of the  
immobilized peptide;

(c) incubating the immobilized peptide under  
conditions conducive to disulfide bond formation;

(d) contacting under conditions which permit  
30 immunospecific binding the body fluid with the  
immobilized peptide to form a reaction mixture;

(e) detecting whether immunospecific binding  
has occurred between the immobilized peptide and an  
antibody component of the body fluid in which the  
35 detection of immunospecific binding indicates the  
presence of antibodies or antigens of the HIV viruses in  
the body fluid.

40. The method of claim 39 wherein immunospecific binding is detected by:

5 (a) removing unbound components from reaction products formed in the immunoreaction mixture;

(b) adding a labeled antibody to the immunoreaction mixture, the labeled antibody being capable of immunospecific binding to a component of the reaction product and the label providing a detectable  
10 signal; and

(c) detecting whether the labeled antibody binds to the reaction products.

41. The method of claim 40 wherein the step of  
15 removing unbound components is by filtration.

42. The method of claim 40, wherein the label is selected from the group consisting of fluorophores, enzymes, luminescent compounds, radioisotopes and  
20 particles.

43. The method of claim 42, wherein the label is an enzyme which is detectable by the addition of an enzyme substrate.  
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44. The method of claim 39, wherein the solid phase is selected from the group consisting of latex, silica, cellulose, fluorocarbon polymers, nylon, polyacrylamide and polystyrene.  
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45. The method of claim 44, wherein the solid phase is selected from the group consisting of latexes of polyacrylamide, polystyrene, silica and cellulose.

35 46. The method of claim 45, wherein the solid phase is a latex of polystyrene.



47. A method for the preparation of an antigen coated solid phase for immunological detection and/or quantitation of antibody to HIV viruses, comprising:

5 (a) synthesizing a peptide composition wherein incorporated cysteine thiol groups are protected by chemically reversible means to form a protected peptide composition;

10 (b) removing said chemically reversible protection means from said protected peptide composition under conditions conducive to intramolecular disulfide bond formation;

(c) immobilizing said peptide composition onto a solid phase to form an antigen coated solid phase.